Featured Article

Immunocytochemical Identification of VPAC<sub>1</sub>, VPAC<sub>2</sub>, and PAC<sub>1</sub> Receptors in Normal and Neoplastic Human Tissues with Subtype-Specific Antibodies

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ABSTRACT

Human tumors frequently overexpress receptors for vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP). However, none of the VIP/PACAP receptor proteins has been visualized individually in human tumors. Here, we developed and characterized a panel of antipeptide antibodies to the carboxyl-terminal regions of the VIP/PACAP receptor subtypes vasoactive intestinal peptide receptor (VPAC<sub>1</sub>), VPAC<sub>2</sub>, and pituitary adenylate cyclase-activating peptide receptor (PAC<sub>1</sub>). Specificity of the antisera was shown by the following: (1) detection of broad bands migrating at Mr 50,000 to 70,000 in Western blots of membranes from receptor-expressing tumors and receptor-transfected cells; (2) cell surface staining of VIP/PACAP receptor-transfected cells; (3) translocation of VIP/PACAP receptor immunostaining in transfected cells after agonist exposure; and (4) abolition of tissue immunostaining by preadsorption of the antibodies with their immunizing peptides. The distribution of VIP/PACAP receptors was investigated in 98 human tumors and their tissues of origin. VPAC<sub>1</sub>, VPAC<sub>2</sub>, and PAC<sub>1</sub> receptors were clearly localized at the plasma membrane of the tumor cells in a variety of human neoplasms. In the gastrointestinal tract, VPAC<sub>1</sub> receptor immunoreactivity was abundant in the mucosa of myenteric neurons; VPAC<sub>2</sub> receptor immunoreactivity was detected in neuroendocrine cells, blood vessels, and smooth muscle; and PAC<sub>1</sub> receptor immunoreactivity was found in myenteric neurons. This is the first localization of all of the VIP/PACAP receptor subtypes in human formalin-fixed, paraffin-embedded tissues. VIP/PACAP receptor visualization with this simple and rapid immunohistochemical method will facilitate identification of tumors with a sufficient receptor overexpression for diagnostic or therapeutic intervention.

INTRODUCTION

Several human tumors overexpress receptors for small regulatory peptides, e.g., somatostatin, bombesin, vasoactive intestinal peptide (VIP), or pituitary adenylate cyclase-activating peptide (PACAP), an observation which has led to a number of clinical applications in the diagnosis and treatment of tumors (1–6). VIP is a 28-amino acid neuropeptide, which is widely distributed throughout the brain and periphery, and which structurally belongs to the glucagon-growth hormone releasing factor-secretin superfamily (7–9). PACAP was first isolated from ovine hypothalamic extracts on the basis of its ability to stimulate cAMP formation in anterior pituitary cells (10). PACAP has recently been shown to exert potent gastrointestinal effects (8, 11). The 175-amino acid pro-PACAP precursor molecule is tissue-specifically processed to either PACAP38 or PACAP27 (8, 11).

The biological actions of VIP and PACAP are mediated by a family of three G protein-coupled receptors, which are designated vasoactive intestinal peptide receptor (VPAC<sub>1</sub>), VPAC<sub>2</sub>, and pituitary adenylate cyclase-activating peptide receptor (PAC<sub>1</sub>) (5, 12, 13). The PAC<sub>1</sub> receptor exhibits lower affinity for VIP than for PACAP, whereas the VPAC<sub>1</sub> and VPAC<sub>2</sub> receptors exhibit similar affinities for VIP and PACAP (5, 12–15). Several observations suggest that VIP/PACAP receptors may be potential targets for tumor imaging and treatment with labeled or unlabeled VIP and PACAP analogs. First, in vitro binding studies have shown that VIP/PACAP receptors are expressed in the great majority of most frequently occurring human tumors, including breast, ovarian, colon, insulinoma, carcinoid, pancreas, glioblastoma, meningioma, pituitary adenoma, and pheochromocytoma (16–22). Second, VIP/PACAP receptor-positive intestinal and endocrine tumors can be visualized by in vivo VIP receptor scintigraphy (1, 4, 6, 23–26). Third, application of VIP and PACAP analogs modulates tumor growth in animal models (2, 3, 27–33).

The expression of VIP/PACAP receptors in human tumors has previously been detected with binding autoradiography or reverse transcription-PCR (16–22). However, the diagnostic value of these methods is limited. The determination of the VIP/PACAP receptor status with in vitro autoradiography in routine tumor diagnostic is costly and time-consuming. Given the limited number of selective ligands, it is also difficult to differentiate between individual VIP/PACAP receptor subtypes. Conversely, with reverse transcription-PCR, it is possible to discriminate between VIP/PACAP receptor subtypes; however, this method is based on total RNA isolation from a fresh tumor...
Table 1  Amino acid sequences of COOH-terminal regions of human VIP/PACAP receptors.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>VPAC1 (438–457)</td>
<td>ATCSTQVSMLTHSVGPSARRSSSFQAVSLY *</td>
</tr>
<tr>
<td>VPAC2 (419–438)</td>
<td>SFNSRSEQALOFHHRSGRAQSLQETSV</td>
</tr>
<tr>
<td>PAC1 (506–525)</td>
<td>SGVNGGQLSLKSSGRMSLPAADNLAT †</td>
</tr>
</tbody>
</table>

* Amino acid sequences of peptides corresponding to COOH-terminal regions used to immunize rabbits for antibody production are underlined.
† This sequence is identical in all known PAC1 splice variants.

The following tumors were investigated: colorectal adenocarcinoma (n = 5); ductal pancreatic adenocarcinoma (n = 5); diffuse type gastric cancer (n = 9); intestinal type gastric cancer (n = 8); hepatocellular carcinomas (n = 10); breast carcinoma (n = 4); ovarian carcinoma (n = 10); prostate cancer (n = 4); thyroid carcinoma (n = 6); carcinoid (n = 15); pancreatic insulinoma (n = 8); pituitary adenoma (n = 4); pheochromocytoma (n = 2); glioblastoma (n = 4); and meningioma (n = 4). Several of the neuroendocrine tumors contained adjacent normal tissue, which was also analyzed. In addition, several fresh tumor specimens were immediately frozen in liquid N2 and stored at −70°C until Western blot analysis. The following tumors were investigated: colorectal carcinoma (n = 6); breast carcinoma (n = 8); ovarian carcinoma (n = 8); thyroid carcinoma (n = 4); pituitary adenoma (n = 4); glioblastoma (n = 8); and meningioma (n = 4).

**Materials and Methods**

**Patients, Tumors, and Tissue Preparation.** Ninety-eight tumor specimens were retrieved from the archives of the Departments of Pathology and Neuropathology. All of the tissue specimens had been fixed in formalin and embedded in paraffin. The following tumors of their tissues of origin.

In the present study, we have generated and characterized a panel of antibodies directed to the COOH-terminal sequences of VIP/PACAP receptor subtypes VPAC1, VPAC2, and PAC1. We have also developed an immunohistochemical protocol that allows efficient detection of these receptors in formalin-fixed, paraffin-embedded human tissues. The generation of these novel antibodies enabled us to determine the cellular distribution of VIP/PACAP receptor proteins in a variety of human tumors and their tissues of origin.

**Materials and Methods**

**Patients, Tumors, and Tissue Preparation.** Ninety-eight tumor specimens were retrieved from the archives of the Departments of Pathology and Neuropathology. All of the tissue specimens had been fixed in formalin and embedded in paraffin. The following tumors were investigated: colorectal adenocarcinoma (n = 5); ductal pancreatic adenocarcinoma (n = 5); diffuse type gastric cancer (n = 9); intestinal type gastric cancer (n = 8); hepatocellular carcinomas (n = 10); breast carcinoma (n = 4); ovarian carcinoma (n = 10); prostate cancer (n = 4); thyroid carcinoma (n = 6); carcinoid (n = 15); pancreatic insulinoma (n = 8); pituitary adenoma (n = 4); pheochromocytoma (n = 2); glioblastoma (n = 4); and meningioma (n = 4). Several of the neuroendocrine tumors contained adjacent normal tissue, which was also analyzed. In addition, several fresh tumor specimens were immediately frozen in liquid N2 and stored at −70°C until Western blot analysis. The following tumors were investigated: colorectal carcinoma (n = 6); breast carcinoma (n = 8); ovarian carcinoma (n = 8); thyroid carcinoma (n = 4); pituitary adenoma (n = 4); glioblastoma (n = 8); and meningioma (n = 4).

**Western Blot Analysis.** Membranes were prepared from stably transfected HEK-293 or CHO cells as well as fresh tumor specimens. Cells and tissues were lysed in homogenization buffer [5 mmol/L EDTA, 3 mmol/L EGTA, 250 mmol/L sucrose, and 10 mmol/L Tris-HCl (pH 7.6)] containing 1 mmol/L phenylmethylsulfonylfluoride, 1 mmol/L pepstatin, 10 μg/mL leupeptin, and 2 μg/mL aprotinin], and membranes were pelleted at 20,000 × g for 30 minutes at 4°C. Membranes were then dissolved in lysis buffer [150 mmol/L NaCl, 5 mmol/L EDTA, 3 mmol/L EGTA, and 20 mmol/L HEPES (pH 7.4)] containing 4 mg/mL dodecyl-β-maltoside and proteinase inhibitors as described above] and incubated with 150 μL wheat germ lectin agarose beads (Amersham) for 90 minutes at 4°C. Beads were washed five times in lysis buffer, and adsorbed glycoproteins were eluted with SDS-sample buffer for 20 minutes at 60°C. When indicated, adsorbed glycoproteins were subjected to enzymatic deglycosylation with peptide N-glycosidase F (PNGase F) according to the manufacturer’s protocol (New England Biolabs, Beverly, MA). Samples were then subjected to 8% SDS polyacrylamide gel electrophoresis and immunoblotted onto nitrocellulose. Blots were incubated with 1 μg/mL anti-VPAC1 (9020), anti-VPAC2 (9023), or anti-PAC1 (9027) antibodies followed by cyanin 3.18-conjugated secondary antibodies (Amersham, Braunschweig, Germany). Specimens were mounted and examined with a Leica TCS-NT laser scanning confocal microscope as described previously (34, 36, 37).

**Immunohistochemistry.** Seven-μm paraffin sections were cut and floated onto positively charged slides and immunohistochemically stained as described previously (34, 38, 39). Briefly, sections were dewaxed, microwaved in 10 mmol/L citric acid (pH 6.0) for 20 minutes at 600 W, and subsequently incubated with 2 μg/mL anti-VPAC1 (9020), anti-VPAC2 (9023), or anti-PAC1 (9027) antibodies overnight at 4°C. Staining of primary antibody was detected with biotinylated goat antirabbit IgG or biotinylated goat antimouse IgG followed by an incubation with avidin-biotinylated peroxidase solution. Tissue was then rinsed and stained with 3,3′-diaminobenzidine-glucose oxidase for 15 minutes. Cell nuclei were lightly counter-
stained with hematoxylin. For immunohistochemical controls, the primary antibody was either omitted, replaced by pre-immune sera, or adsorbed with several concentrations ranging from 1 to 10 μg/mL of homologous or heterologous peptides for 2 hours at room temperature. A tumor known to stain positively was included in each batch of staining as a positive control.

RESULTS
Characterization of VIP/PACAP Receptor Antibodies. Specificity of the antisera was monitored with Western blot analysis. When membrane preparations from stable transfected cells were electrophoretically separated and blotted onto nitrocellulose, the antisera 9020 (anti-VPAC1), 9023 (anti-VPAC2), and 9027 (anti-PAC1) revealed broad bands in cells transfected with their cognate VIP/PACAP receptor subtype (Fig. 1). In HEK-293 cells stably expressing VPAC1, the anti-VPAC1 antibody (9020) detected a broad band migrating at $M_r$ 55,000 to 70,000 (Fig. 1A). In HEK-293 cells stably expressing VPAC2, the anti-VPAC2 antibody (9023) detected a single broad band migrating at $M_r$ 50,000 to 65,000 (Fig. 1B). In CHO cells stably expressing PAC1, the anti-PAC1 antibody detected a broad band migrating at $M_r$ 40,000 to 60,000 (Fig. 1C). Enzymatic deglycosylation with PNGase F resulted in shift of molecular size and appearance of all three VIP/PACAP receptor proteins (Fig. 1).

Antisera were additionally characterized with immunofluorescent staining of transfected cells. When HEK-293 cells stably expressing VPAC1 or VPAC2 and CHO cells stably expressing PAC1 were stained with anti-VPAC1 (9020; A), anti-VPAC2 (9023; B), or anti-PAC1 (9027; C) antibodies, prominent immunofluorescence localized at the level of the plasma membrane was detected (Fig. 2, A, C, and E). After incubation with VIP or PACAP27, VPAC1-, VPAC2-, and PAC1-immunoreactivity was translocated from the plasma membrane into the cytosol, indicating that all of the VIP/PACAP receptor subtypes were rapidly endocytosed in an agonist-dependent manner (Fig. 2, B, D, and F). Whereas VPAC1 and VPAC2 were internalized in response to both VIP and PACAP27, PAC1 was selectively internalized in response to PACAP27 (data not shown). Next, the antisera were tested for possible cross-reactivity with other proteins.
VPAC1, VPAC2, and PAC1 Receptors in Human Tissues

Western blot analysis of the specificity of anti-VPAC1, anti-VPAC2, and anti-PAC1 antibodies in human tumors. A. Membrane preparations from an ovarian carcinoma were separated on an 8% SDS-polyacrylamide gel, blotted onto nitrocellulose, and incubated with 1 μg/mL anti-VPAC1 antibody (9020) in the absence (−) or presence (+) of 10 μg/mL peptide antigen. B. Membrane preparations from a breast carcinoma were separated on an 8% SDS-polyacrylamide gel, blotted onto nitrocellulose, and incubated with 1 μg/mL anti-VPAC2 antibody (9023) in the absence (−) or presence (+) of 10 μg/mL peptide antigen. C. Membrane preparations from a glioblastoma were separated on an 8% SDS-polyacrylamide gel, blotted onto nitrocellulose, and incubated with 1 μg/mL anti-PAC1 antibody (9027) in the absence (−) or presence (+) of 10 μg/mL peptide antigen. Blots were developed with enhanced chemiluminescence. Representative results from one of five independent experiments are shown. Ordinate, migration of protein molecular weight markers (Mr × 10^3).

Fig. 3 Western blot analysis of the specificity of anti-VPAC1, anti-VPAC2, and anti-PAC1 antibodies in human tumors. A–C. Membrane preparations from a glioblastoma were separated on an 8% SDS-polyacrylamide gel, blotted onto nitrocellulose, and incubated with 1 μg/mL anti-VPAC1 antibody (9020) in the absence (−) or presence (+) of 10 μg/mL peptide antigen. B. Membrane preparations from a breast carcinoma were separated on an 8% SDS-polyacrylamide gel, blotted onto nitrocellulose, and incubated with 1 μg/mL anti-VPAC2 antibody (9023) in the absence (−) or presence (+) of 10 μg/mL peptide antigen. C. Membrane preparations from a glioblastoma were separated on an 8% SDS-polyacrylamide gel, blotted onto nitrocellulose, and incubated with 1 μg/mL anti-PAC1 antibody (9027) in the absence (−) or presence (+) of 10 μg/mL peptide antigen. Blots were developed with enhanced chemiluminescence. Representative results from one of five independent experiments are shown. Ordinate, migration of protein molecular weight markers (Mr × 10^3).

V1/V2/PAC1 Receptor Immunohistochemical Staining in Normal and Neoplastic Human Tissues. The anti-VPAC1 (9020), anti-VPAC2 (9023), and anti-PAC1 (9027) antibodies were subjected to immunohistochemical staining of a variety of human tissues. Initial experiments showed that heat-induced epitope retrieval is required for efficient immunohistochemical staining of paraffin-embedded tissue (data not shown). Many neuroendocrine tumors contained adjacent noncancerous tissue, which enabled us to analyze the distribution of VIP/PACAP receptors in several parts of the normal gastrointestinal tract. Prominent localizations of VPAC1, VPAC2, and PAC1 receptors in the small intestine are shown in Fig. 4. VPAC1 receptor immunoreactivity was abundant in the mucosa (Fig. 4A). The highest densities of immunoreactive VPAC1 receptors were observed in epithelial cells located in the basal portion of the crypts (Fig. 4A). In epithelial cells, VPAC1 receptor immunoreactivity was predominantly confined to the plasma membrane (Fig. 4B). In contrast, VPAC2 receptor immunoreactivity was not seen in epithelial cells but selectively localized to neuroendocrine cells (Fig. 4C). In neuroendocrine cells, immunoreactive VPAC2 receptors were distributed throughout the cytosol (Fig. 4C). VPAC2 receptor immunoreactivity was also seen in smooth muscle of the gastrointestinal tract and blood vessels (Fig. 4D). VPAC1 receptor immunoreactivity was observed in myenteric neurons as well as in fibers distributed to the muscle layer (Fig. 4E). In addition, VPAC1 receptor immunoreactivity was abundant in neurons of the mucosal plexus (data not shown). PAC1 receptor immunoreactivity was also observed in many neurons of the myenteric plexus (Fig. 4F). The anti-VPAC1 (9020), anti-VPAC2 (9023), and anti-PAC1 (9027) antibodies were then subjected to immunohistochemical staining of 98 human tumors. All three antibodies yielded prominent staining predominantly localized to the plasma membrane of the tumor cells (Fig. 5). The staining intensity for each antibody varied greatly between individual tumors giving consistently different sample-specific patterns of VIP/PACAP receptor subtype expression under otherwise identical conditions. Immunostaining for each antiserum was completely abolished by preadsorption with 10 μg/mL of the immunizing peptides (Fig. 5). Table 2 summarizes the incidence of VPAC1, VPAC2, and PAC1 receptors in a series of human tumors, including some of the most frequently occurring carcinomas. The VPAC1, VPAC2, or PAC1 receptors were found in every tumor except thyroid cancer, which was completely devoid of any of the VIP/PACAP receptors (Table 2). The VPAC1 receptor was found most commonly in cancers of the breast, pancreas, and prostate, as well as in pituitary adenomas. The VPAC2 receptor was commonly expressed in meningiomas and less frequently in carcinoids, insulinomas, gastric cancers, and breast cancers. The VPAC2 receptor was also found in a single pheochromocytoma. The PAC1 receptor was most commonly expressed by neuroendocrine tumors, meningiomas, and breast cancer, and was rarely found in cancers of the stomach, liver, colorectum, thyroid, or prostate. A uniform highly abundant expression of all of the VIP/PACAP receptor subtypes was only evident in glioblastomas (Table 2). VPAC1, VPAC2, and PAC1 were also present at the plasma membrane of neuronal cell bodies and processes adjacent to the glioblastomas (data not shown).

DISCUSSION

In an effort to study the pattern of VIP/PACAP receptor protein expression in normal and neoplastic human tissues, we generated antibodies that exert selective specificity for VPAC1, VPAC2, and PAC1 receptors. We show that the cytoplasmic tails of these receptors can serve as an epitope for the generation of antisera that effectively stain formalin-fixed, paraffin-embedded human tissues. Several lines of evidence indicate that these antibodies specifically detect their targeted VIP/PACAP receptor and do not cross-react. First, in Western blots of membranes from receptor-transfected cells, the anti-VPAC1, anti-VPAC2, and anti-PAC1 antibodies detected broad bands migrating at Mr 50,000 to 70,000. Second, anti-VPAC1, anti-VPAC2, and anti-PAC1 antibodies revealed prominent cell surface staining of VIP/PACAP receptor-transfected cells. This immunostaining...
translocated from the cell surface into the cytosol after agonist exposure, indicating rapid endocytosis of all three VIP/PACAP receptor subtypes. Third, in Western blots of membranes from receptor-expressing tumors, the anti-VPAC1, anti-VPAC2, and anti-PAC1 antibodies detected single bands migrating at the appropriate molecular weight. Fourth, tissue immunostaining of all three antisera was completely abolished by preadsorption with homologous but not heterologous peptides. Finally, it should be noted that two of three VPAC1, three of three VPAC2, and two of three PAC1 antisera gave similar results.

The availability of subtype-specific VIP/PACAP receptor antibodies will facilitate additional basic morphologic investigation of VPAC1, VPAC2, and PAC1 receptor expression in human tumors and normal human tissues. The immunohistochemical VIP/PACAP receptor evaluation offers several major advantages. This new method can analyze VIP/PACAP receptors in routinely processed archival paraffin-embedded material of any diagnostic pathology center. It requires only an immunopathological laboratory to perform the test, which can be carried out without costly and time-consuming receptor autoradiography. The immunohistochemical evaluation of the complete VIP/PACAP receptor status of a given tumor specimen can be accomplished in <24 hours. Although one could expect the immunohistochemical VIP/PACAP receptor determination to be less sensitive than receptor autoradiography with 125I-labeled VIP, the sensitivity of the anti-VPAC1 (9020), anti-VPAC2 (9023), and anti-PAC1 (9027) antibodies generated and characterized in this study was sufficiently high enough to detect these receptors in known human VIP/PACAP target tissues, i.e., detection of VPAC1 in gastrointestinal mucosa and VPAC2 in smooth muscle of the gastrointestinal tract. In addition, the immunohistochemical VIP/PACAP receptor determination provided a better histologic quality and cellular resolution compared with receptor autoradiography. Thus, previously unappreciated cellular localizations of VIP/PACAP receptors were uncovered, i.e., presence of VPAC1 in myenteric nerve fibers and VPAC2 in neuroendocrine cells.

Although VPAC1, VPAC2, and PAC1 were all highly abundant throughout the gastrointestinal tract, the various VIP/PACAP receptor subtypes exhibited complementary expression patterns. Previous studies have shown that VIP immunoreactivity is present in neuroendocrine cells as well as in submucous and myenteric ganglia of the human gut (40–42). VIP immunoreactivity is also present in mucosal and myenteric nerve fibers as well as in nerve fibers innervating blood vessels (40–42). PACAP immunoreactivity exhibits a nearly identical distribution (40–42). In fact, dual immunofluorescence studies revealed a high degree of coexistence of the two peptides (40–42). Thus, the present findings suggest distinct roles of individual VIP/PACAP receptor subtypes in gastrointestinal physiology. VPAC1 in the mucosa is targeted by VIP and PACAP released from mucosal nerve fibers and neuroendocrine cells and is possibly involved in the regulation of secretion. VPAC2 in the smooth muscle of the gastrointestinal wall is

![Fig. 4 VPAC1, VPAC2, and PAC1 immunohistochemical staining in the normal gastrointestinal tract. Sections were dewaxed, microwaved in citric acid, and incubated with affinity-purified anti-VPAC1 (9020; A, B, and E), anti-VPAC2 (9023; C and D), or anti-PAC1 (9027; F) antibodies at a concentration of 2 μg/mL. Sections were then sequentially treated with biotinylated antirabbit IgG and AB solution. Sections were then developed in 3,3’-diaminobenzidine–glucose oxidase and lightly counterstained with hematoxylin. Note that in the small intestine, VPAC1 receptor immunoreactivity was abundant in the mucosa and myenteric neurons, VPAC2 receptor immunoreactivity was detected in neuroendocrine cells and smooth muscle of blood vessels, and PAC1 receptor immunoreactivity was seen in myenteric neurons. Representative results from one of three independent experiments are shown. Scale bar: A = 200 μmol/L; B–F = 100 μm.](image-url)
targeted by VIP and PACAP released from myenteric nerve fibers and is probably involved in the regulation of motor activity. Conversely, VPAC2 in the smooth muscles of blood vessels may regulate gastrointestinal blood flow. VPAC1 in myenteric nerve fibers is likely to function as autoreceptor, regulating the release of VIP and PACAP. VPAC2 in neuroendocrine cells may also function as autoreceptor, which may explain the high levels of internalized receptors detected in these cells.

<table>
<thead>
<tr>
<th>Tumor type (n)</th>
<th>VPAC1 n (%)</th>
<th>VPAC2 n (%)</th>
<th>PAC1 n (%)</th>
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<tr>
<td>Gastric cancer, diffuse type (9)</td>
<td>1 (11) *</td>
<td>2 (22) *</td>
<td>0</td>
</tr>
<tr>
<td>Gastric cancer, intestinal type (8)</td>
<td>4 (50) *</td>
<td>5 (62) *</td>
<td>0</td>
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<tr>
<td>Colorectal adenocarcinoma (5)</td>
<td>4 (80)</td>
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<td>2 (40)</td>
</tr>
<tr>
<td>Pancreatic ductal adenocarcinoma (5)</td>
<td>5 (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hepatocellular carcinoma (10)</td>
<td>4 (40) *</td>
<td>3 (30) *</td>
<td>0</td>
</tr>
<tr>
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<td>2 (50)</td>
<td>4 (100)</td>
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<tr>
<td>Ovarian carcinoma (10)</td>
<td>3 (30)</td>
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<td>3 (30)</td>
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<tr>
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<td>3 (75)</td>
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<td>0</td>
<td>0</td>
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<td>6 (40)</td>
<td>8 (33)</td>
<td>8 (33)</td>
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<td>3 (38)</td>
<td>4 (50)</td>
<td>3 (38)</td>
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<tr>
<td>Pituitary adenoma, non-functional (2)</td>
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<td>0</td>
<td>2 (100)</td>
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<tr>
<td>Pheochromocytoma (2)</td>
<td>0</td>
<td>1 (50)</td>
<td>2 (100)</td>
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<td>Glioblastoma multiforme (4)</td>
<td>4 (100)</td>
<td>4 (100)</td>
<td>4 (100)</td>
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<tr>
<td>Meningioma (4)</td>
<td>2 (50)</td>
<td>4 (100)</td>
<td>3 (75)</td>
</tr>
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</table>

* <10% of the tumor cells showed membranous expression of either VPAC1, VPAC2, or PAC1.
cells. Given that PAC1 has a higher affinity for PACAP than for VIP, it is conceivable that myenteric neurons bearing PAC1 are selectively responsive to PACAP.

VIP and PACAP can affect the growth of human tumors cells in vivo and in vitro (2, 3, 27–33). Whereas growth-promoting activities have been reported for VIP, growth-inhibiting properties have been found for VIP antagonists in various tumor models (2, 3, 27–33). VIP/PACAP receptors are detectable in the great majority of most frequently occurring human tumors, including breast, ovarian, colon, insulinoma, carcinoid, pancreas, glioblastoma, meningioma, pituitary adenoma, and pheochromocytoma. It is therefore very tempting to suggest the use of growth-inhibiting VIP/PACAP analogs for the treatment of human tumors (16–22). Moreover, a number of clinical and preclinical studies suggest that VIP/PACAP receptors may be promising molecular targets for tumor imaging and targeted radiotherapy (1, 4, 6, 23–26). However, previous and present evidence indicates a highly abundant expression of VIP/PACAP receptors in normal human target tissues. Thus, for potential clinical application of VIP/PACAP analogs, the ratio of receptor expression in neoplastic versus normal tissues should be determined with subtype-specific VIP/PACAP receptor antibodies.

In conclusion, we have generated and extensively characterized anti-VPAC1, anti-VPAC2, and anti-PAC1 antibodies. Using these antibodies, we provide the first demonstration of all of the VIP/PACAP receptor subtypes in human formalin-fixed, paraffin-embedded tissues. It is now possible to determine the exact cellular and subcellular sites of VIP/PACAP receptor protein expression in normal human tissues, which is needed for a better understanding of VIP/PACAP actions in physiologic target tissues. The rapid immunocytochemical VIP/PACAP receptor visualization may also be helpful to identify those tumors with a sufficient receptor overexpression for diagnostic or therapeutic intervention.

ACKNOWLEDGMENTS

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